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In vitro evaluation of native bacterial antagonists against *Fusarium oxysporum* f. sp. *ciceri* causing wilt of chickpea

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Abstract
Chickpea (*Cicer arietinum* L.) is a major *Rabi* pulse crop in India. Among various diseases, wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is major devastating chickpea disease that causes yield losses of 10 to 90 percent in severe cases. As it is both soil and seed borne, difficult to control by using fungicides. Hence a study was undertaken to determining efficacy of twenty native bacterial antagonists were evaluated against *Fusarium oxysporum* f. sp. *ciceri*. Among Bacillus, isolate BRSN-B2 proved best with 57.93 percent growth inhibition, while *Pseudomonas fluorescence* isolate CRSN-PF1 was effective showing 50.74 percent inhibition of the pathogen.

Keywords: Chickpea, *Fusarium*, bacillus, *Pseudomonas fluorescence* & wilt

Introduction
Chickpea (*Cicer arietinum* L.) is a highly self-pollinated crop of family Fabaceae with diploid chromosome number 2n=16 (Arumuganathan and Earle, 1991; Anonymous, 2019) [2, 1]. It is also known as gram or Bengal gram, garbanzo or garbanzo bean, or Egyptian pea. Chickpea contains dietary fibers, proteins, vitamins, and minerals etc. Chickpea consists of 9 annual and 34 perennial wild species. Among the 9 annual species, chickpea (*C. arietinum* L.) is the only cultivated species. Chickpea is a cool season, drought hardy grain legume crop having majority of its cultivation in dry areas of the Indian subcontinent (Saxena, 1990) [12].
Wilt disease caused by *F. o.* f.sp. *ciceri* is one of the important and of common occurrences, whenever chickpea crop is grown. It is the major soil-borne fungus affecting chickpeas around the globe (Kraft *et al.*, 1994) [9]. So far, eight pathogenic races of Foc (races 0, 1A, 1B/C, 2, 3, 4, 5 and 6) have been reported throughout the world (Haware and Nene, 1982; Jendoubi *et al.*, 2017) [3, 5]. The pathogen is mainly soil borne (Jimenez-Fernandez *et al*., 2011) [6] as well as seed borne (Pande *et al*., 2007) [10] and it is a facultative saprophyte (Haware *et al*., 1986) [4]. The management of plant pathogens in soil with fungicides has been creating problems of fungi resistance, ecosystem imbalance by toxic effects of residues and animal health hazards. Biocontrol agents do not harm the environment and have proven themselves to be a cheap alternative to harmful chemical pesticides, and they also do not need to be ingested by the host but can invade them directly. So, individual as well as combined effects of some rhizobacterial isolates might be helpful to develop suitable strategies to reduce infection of some root pathogens in chickpea.

Materials and Methodology
The antagonistic activity of isolated rhizospheric bacterial bio agents against Fusarium wilt of chickpea was determined by dual culture technique under *in vitro* condition using completely randomized design (CRD). Mycelial disc of 5 mm diameter cut from the margin of 5 days old culture of test pathogen and streak of bacterial antagonists were placed opposite to each other on PDA in Petri plates (90 mm). The petri plates with disc of *Fusarium* alone were served as the control. The inoculated petri plates were incubated at 27±2 °C in BOD incubator. After the completion of incubation period the growth of *Fusarium oxysporum* f. sp. *ciceri* was measured and the percent growth inhibition of intersecting colonies was calculated.

Result and Discussion

A total of twenty native bacterial antagonist isolates were evaluated *in vitro* against *F. oxysporum* f.sp. *ciceri* which exhibited a wide range of mycelial growth and inhibition of the test pathogen.

Radial Mycelial growth

The radial mycelial growth exhibited by the test pathogen (*F. oxysporum* f. sp. *ciceri*) from 37.87 mm (BRSN-B2) to 69.50 mm (CRSDN-PF1) growth as against 90.00 mm in untreated control. However, BRSN-B2 was found best with least mycelial growth (37.87 mm) followed by CRSN-PF1 (44.33 mm). This treatment followed by BRSN-B1 (48.77 mm), CRSN-B2 (50.03 mm) and BRSN-PF2 (52.50 mm). Amongst all the tested native bacterial antagonist isolates, CRSDN-PF1 was found comparatively less effective with maximum mycelial growth of 69.50 mm.

Mycelial growth inhibition

Percent mycelial growth inhibition of the test pathogen was ranged from 22.78 percent (CRSDN-PF1) to 57.93 percent (BRSN-B2). However, BRSN-B2 was found best with highest mycelial growth inhibition (57.93%) followed by CRSN-PF1 (50.74%). This treatment followed by BRSN-B1 (45.81%), CRSN-B2 (44.41%) and BRSN-PF2 (41.67%). Amongst all the tested native bacterial antagonist isolates, CRSDN-PF1 was found comparatively less effective with least mycelial growth inhibition (22.78%).

However, Bacillus spp. Isolate BRSN-B2 showed maximum percent growth inhibition (57.93%) and with least mycelial growth (37.87 mm).

Similar growth inhibition of the test pathogen *F. oxysporum* f.sp. *ciceri* under *in-vitro* condition was observed by several workers Keote *et al.* (2019) [8] reported that *P. fluorescens* inhibited fungal growth to the extent of 55.31 percent, followed by *B. subtilis* with 48.22 percent and radial growth of the Foc in *P. fluoresce was* 55.31mm and in *B. subtilis* 48.22 mm. similarly Kapali *et al*. (2016) [7] disclosed that radial mycelial growth of Foc with *P. fluorescens* was ranging from 35.66 to 81.33 mm and percent inhibition ranging from 9.63 to 60.37 percent and radial mycelial growth of Foc with *B. subtilis* was ranging from 28.33 to 78.87 mm and percent inhibition ranging from 12.36 to 68.52 percent. According to Trivedi *et al.*, (2020) [13] *B. subtilis* and *P. fluorescens* caused 60.0 and 68.6 percent reduction in mycelial growth of the pathogen, over control.

Table 1: *In vitro* bio-efficacy of native bacterial antagonists against *Fusarium* wilt of chickpea

Treatment	Isolates	Colony Dia.* (mm)	Percent inhibition of mycelia growth
T_1	CRSI-PF1	68.83	23.52 (29.01)#
T_2	CRSI-PF2	69.17	23.15 (28.74)
T_3	CRSN-PF1	44.33	50.74 (45.42)
T_4	CRSN-PF2	56.17	37.59 (37.81)
T_5	BRSI-PF1	68.50	23.89 (29.26)
T_6	BRSN-PF1	60.17	33.15 (35.14)
T_7	BRSN-PF2	52.50	41.67 (40.20)
T_8	CRSDI-PF1	68.87	23.48 (28.98)
T_9	CRSDI-PF2	68.57	23.81 (29.21)
T_{10}	CRSDN-PF1	69.50	22.78 (28.50)
T_{11}	CRSDN-PF2	55.20	38.67 (38.44)
T_{12}	CRSI-B1	53.03	41.07 (39.86)
T_{13}	CRSN-B1	52.80	41.33 (40.01)
T_{14}	CRSN-B2	50.03	44.41 (41.79)
T_{15}	BRSI-B1	52.17	42.04 (40.41)
T_{16}	BRSN-B1	48.77	45.81 (42.60)
T_{17}	BRSN-B2	37.87	57.93 (49.56)
T_{18}	CRSDI-B1	57.23	36.41 (37.11)
T_{19}	CRSDN-B1	52.43	41.74 (40.20)
T_{20}	CRSDN-B2	50.50	43.89 (41.49)
T_{21}	Control	90.00	0.00 (0.00)
SE±		0.92	1.02
CD @ 1%		4.95	2.95

*Mean of three replications, Dia. =Diameter,
(# Figures in parenthesis are arc sine transformed value)

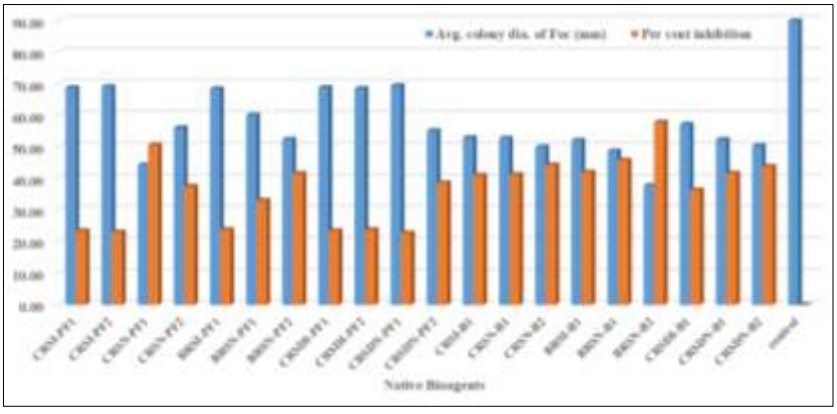

Fig 1: *In vitro* bio-efficacy of native bacterial antagonists against *Fusarium* wilt of chickpea

Plate 1: Dual culture of native bacterial antagonists against *Fusarium oxysporum* f.sp. *ciceri*

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